1890-Pos
The Non-Equilibrium Thermodynamics and Kinetics of Focal Adhesion Dynamics
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We consider a focal adhesion to be made up of molecular complexes, each consisting of a ligand such as fibronectin, an integrin molecule, and associated plaque proteins. Free energy changes drive the binding and unbinding of these complexes and thereby control the focal adhesion's dynamic modes of growth, treadmilling and resorption. The free energy changes include mechanical and chemical contributions. We have identified a competition among four thermodynamic driving forces for focal adhesion dynamics: (1) the work done during the addition of a single molecular complex of a certain size, (2) the chemical free energy change associated with the addition of a molecular complex, (3) the elastic free energy change associated with deformation of focal adhesions and the cell membrane, and (4) the work done on a molecular conformational change. We have developed a theoretical treatment of focal adhesion dynamics as a nonlinear rate process governed by a classical kinetic model, and demonstrate the limitations of the associated linear response theory. We also express the rates as being driven by out-of-equilibrium thermodynamic driving forces, and modulated by kinetics. In the resulting model, the mechanisms governed by the four effects described above allow focal adhesions to exhibit a rich variety of behavior without the need to introduce special constitutive assumptions for their response. The reaction-limited case is considered. Our central findings are that growth, treadmilling and resorption are all predicted by a very simple chemo-mechanical model. Treadmilling requires symmetry breaking between the ends of the focal adhesion, and is achieved by driving force (1) above. In contrast, depending on its numerical value (2) causes symmetric growth, resorption or is neutral, (3) causes symmetric resorption, and (4) causes symmetric growth.

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Effect of Emodin on Traction Force of Breast Cancer Cells
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Tumor cells sense, process and respond to biological, chemical and mechanical cues in their environment. Regardless of the type of stimuli, the biochemical output of the cells is well studied, from phenotypic changes to protein and gene expression. However, the mechanical output from cells (other than cell elasticity) has not been emphasized. In this study, we have demonstrated the effect of emodin (an anti-cancer drug) on human breast cancer cells in terms of their mechanical response, cytoskeleton changes and the expression of adhesion-related structures. Emodin causes the cell traction forces to increase due to cell rounding and the associated pulling of the ECM which are attributable to emodin's anti-adhesive effect. The traction forces may then decrease and these lower forces depict the compromised well-being of the apoptosizing cells. The variations in traction forces were correlated to morphological and cytoskeletal changes. Such mechano-chemical relationships also reveal cell-matrix interactions and the results have significant implications for understanding relationships which can reveal cell-matrix interactions and help in the quantitative assessment of drug efficacy.

1892-Pos
Substrate Contributions in Elastic Pillar Arrays: Correction of Cellular Force Measurements
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The generation of tensile forces by cells is crucial for adhesion, migration, and cell to cell communication. The magnitude and direction of attachment forces are routinely measured by the deflection of elastic pillars on which the cells have been cultured. As the experimental calibration of the pillar spring constant is tedious, many studies use bulk material properties plus pillar dimensions for the force calculations and consider only bending and shear of the pillar. In this paper we show that all models that neglect the elastic substrate beneath the pillar systematically overestimate the applied forces, typically by more than 20%. Using finite element simulations we find that the elastic substrate accounts for 10-35% of the total deflection at the pillar top. The additional contribution arises from substrate warping at the pillar base and a consequent tilting of the pillar axis. The theoretical findings were verified by experiments with a macroscopic pillar model. We derive an analytical formula which can be used to correct the force calculations for given material properties and pillar aspect ratio. We further investigate the substrate-mediated crosstalk between pillars. We find that the force-loaded pillar acts as a force dipole and the coupling of a second pillar can be described in the framework of its dipole field. The crosstalk diminishes with the third power of the pillar-to-pillar distance and is under the detection limit for most practical cases. Our results highlight the importance of correcting for the systematic errors when comparing cellular forces that were derived from pillar arrays with different dimensions.

1893-Pos
High Resolution Force Distribution of Migrating and Spreading Cells
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Cell motility is crucial for the immune system and its misregulation is associated with cancer and other diseases. Migration is a complex cellular activity with many proteins coordinating within a subcellular motility machine. The machine's parts conspire to propel the cell forward by exerting forces on the environment with spatial variations down to the nanoscale. In this work we measured and statistically characterized the force distributions with the aim of illuminating underlying mechanisms. We cultured mouse embryonic fibroblast cells expressing GFP-actin on dense arrays of flexible nanoengineered pillars. During cell spreading pillar deflections quantitatively revealed cell traction patterns on the substrate. Simultaneous actin imaging enabled the cellular force map to be visualized within the evolving cell contour. The greatest forces were exerted in a ~10-20 micron strip behind the leading edge and are directed inward. We analyzed time profiles of forces exerted on single pillars as the cell passed over the pillar location. Forces were relatively short lived and showed more fluctuation for pillars with smaller diameter than for larger ones. When the protruding cell edge reaches a pillar a period of large traction force begins, typically lasting several minutes, with maximum stress on the order of several nN/μm2. Stiffer substrates provoked larger tractions than more compliant substrates. Once the front of the cell has passed by, a drastic reduction in traction occurs. Spatial force distribution and its evolution were characterized by correlation functions relative to the leading edge. By varying the geometric parameters of the pillars, we probed a range of effective substrate rigidities. In summary, we combined high resolution measurements, statistical analysis and quantitative modeling to characterize and interpret signature features of migrating and spreading cell force maps.

1894-Pos
Oxldl-Induced Decrease in Lipid Order of Membrane Domains is Inversely Correlated with Endothelial Stiffness and Network Formation
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Oxidized Low Density Lipoprotein (OxLDL) is a major factor in development of atherosclerosis. Our earlier studies have shown that exposure of endothelial cells (EC) to oxLDL increases EC stiffness, facilitates the ability of the cells to generate force and facilitates EC networks formation in 3D collagen gels. In this study, we show that oxLDL induces a decrease in lipid order of membrane domains and that this effect is inversely correlated with endothelial stiffness, contractility and network formation. Local lipid packing of cell membrane domains is assessed by Laurdan two-photon imaging, endothelial stiffness was assessed by measuring cellular elastic modulus using Atomic Force Microscopy (AFM), cell contractility was estimated by measuring the ability of the cells to contract collagen gels and EC angiogenic potential was estimated by visualizing endothelial networks within the same gels. Furthermore, we also show that the impact of oxLDL on endothelial biomechanics and network formation is fully reversed by supplying the cells with a surplus of cholesterol suggesting that changes in membrane cholesterol underlie oxLDL-induced effects on endothelial biomechanics. In contrast, exposure to sphingomyelinase C (SMase C) has no effect on endothelial stiffness and network formation, indicating that hydrolysis of sphingomyelin cannot be responsible for these effects. Based on these observations, we suggest that disruption of lipid packing of cholesterol-rich membrane domains plays a key role in oxLDL-induced changes in endothelial biomechanics.